Evidence-based Practice Center Systematic Review Protocol

Project Title: Omega 3 Fatty Acids and Cardiovascular Disease -- Update

I. Background and Objectives for the Systematic Review

The Office of Dietary Supplements (ODS), the National Institutes of Health (NIH), has a long history of commissioning AHRQ-based systematic reviews and research methodology reports for nutrient-related topics (http://ods.od.nih.gov/Research/Evidence-Based_Review_Program.aspx). Omega-3 fatty acids (n-3 FA) and their potential relationship to a broad range of health outcomes formed the basis for nine of these systematic reviews published between 2004 and 2006 and also served as examples for several methodological reports (1-14).

The purpose of the current systematic review is twofold: a) to update an earlier review of the state-of-the science on the topic of the effects of n-3 FA on cardiovascular disease (CVD) (15), and b) to use this new review to collect additional information that would enhance the usefulness of this report for policy and clinical applications.

Since the publication of the original n-3 FA systematic reviews in the mid-2000s the topic of n-3 FA and health has remained controversial and dynamic. This topic has been evaluated by several expert panels as they were considering whether recommendations or reference values for intakes of n-3 FA were warranted, either through naturally occurring sources of n-3 FA (e.g., fish consumption) and/or through the use of dietary supplements and fortified foods (16-19). The n-3 FA (including alpha-linolenic acid [ALA], stearidonic acid [SDA], eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], and docosahexaenoic acid [DHA]) are a group of long chain polyunsaturated fatty acids that serve as precursors for bioactive compounds such as eicosanoids and are integral components of cell membranes. In 2002, the Institute of Medicine (IOM) considered the evidence inadequate to establish an estimated average requirement (EAR) for n-3 FA. Thus the IOM only established adequate intake values (AIs) for ALA, based on current population ALA intake and an apparent absence of deficiency symptoms. For healthy adults, AIs for ALA are 1.1 g/d for females and 1.6 g/d for males (16). After evaluating evidence linking the very long chain n-3 FA—EPA and DHA—to coronary heart disease and stroke, the IOM panel suggested that n-3 FA may provide beneficial health effects with respect to coronary heart disease and stroke when consumed at levels ranging from 0.6% to 1.2% of energy (roughly equivalent to 1 to 3 g/d) (16) (Note that SDA and DPA have only infrequently been analyzed in regards to their association with CVD). Three other expert reports evaluated the potential health benefits of fish/seafood consumption (17-19). Based primarily on the availability of observational study data, these panels consistently suggested that regular consumption of fish and seafood is associated with lower risk of coronary heart disease and cardiac death.

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These recommendations were based primarily on assumptions of benefits from EPA and DHA and their content in fish and seafood.

There are ongoing concerns in the scientific community regarding systematic biases and random errors in the determination of intakes of n-3 FA from dietary and supplement sources using currently available assessment tools. The limitations of the current methods have been discussed elsewhere (20,21, 22). To date, no alternate methods are available. Until "error-free" or "bias-free" methodologies are developed, it is crucial to evaluate the available data with these methodological quality and limitations in mind. Nutrient biomarkers can provide an objective measure of dietary status. However, the correspondence between intake and biomarker concentration not only reflects recent intake but subsequent metabolism (e.g., elongation, desaturation, metabolism to bioactive compounds). Current biomarkers used to estimate n-3 FA intake include ALA, EPA, DHA, and, less frequently, SDA and DPA measured in adipose tissue, erythrocytes, plasma or plasma phospholipids (23,24). Adipose tissue FA are thought to reflect long-term intake, erythrocytes FA are thought to reflect the previous 120 day intake, and plasma FA are thought to reflect more immediate intake (24).

Several recent systematic reviews of randomized controlled trials (RCTs) in individuals and patients with diagnosed CVD or at high risk of CVD have suggested mixed results as to whether there are benefits of very long chain polyunsaturated fatty acids (EPA and DHA) for reducing the risk of adverse cardiovascular outcomes (12,25-31). Reasons for the apparent inconsistent scientific conclusions among several of the expert panels and the more recent systematic reviews are varied but may relate, in part, to whether the n-3 FA exposures were from fish (or other marine) or plant sources or from dietary supplements. The expert reviews also vary as to whether they relied primarily on observational studies or RCTs (12,25-31). Studies of different designs each have their own strengths and weakness that may result in differences in conclusions. For example, observational studies based on self-reported dietary assessments (e.g., food frequency questionnaires) may inaccurately estimate n-3 FA intake; RCTs of specific fish or other n-3 FA rich food may impose an artificial dietary pattern that might not be applicable to the general population; RCTs of supplements might not fully account for differences in background n-3 FA intake; studies using either study design may have subtle differences in eligibility criteria, e.g., length of follow-up period, or inclusion of ALA, EPA and DHA or only EPA and DHA, that significantly impacted the final conclusions. Therefore, it is of interest to systematically compare results across different exposure/intervention products and different study types (e.g., interventional vs. prospective cohort studies), and to account for differences in background n-3 FA intake. Also of interest is a systematic evaluation of possible reasons for inconsistencies between observational and RCT findings (32), in particular a tabulation of causality-related study features.

The 2004 review screened about 7,500 abstracts and retrieved and screened 768 full text articles for potentially relevant human data. Eleven RCTs and one prospective cohort study reported outcomes in individuals with diagnosed CVD. Twenty-two prospective cohort studies and one RCT reported data on general populations. This review will update the previous review for the outcomes included and will also expand the scope to include additional CVD outcomes (peripheral vascular disease, congestive heart failure, and arrhythmias), and will also update the 2004 review of cardiovascular

risk factors and intermediate markers of CVD (34)—specifically blood pressure and plasma lipids—and biomarkers of n-3 FA intake.

II. The Key Questions

The key questions address both issues of efficacy (i.e., causal relationships from trials) as well as associations (i.e., prospective cohort study results and outcomes or risk factors from RCTs for which the randomization may not be applicable). Compared with the key questions from the 2004 reports, they expand the scope of the review to include additional cardiovascular outcomes (peripheral blood pressure, congestive heart failure, arrhythmias, and hypertension), focus on the intermediate outcomes plasma lipids and blood pressure, and include associations between biomarkers of intake and outcomes.

1. What is the efficacy or association of n-3 FA (EPA, DHA, EPA+DHA, DPA, SDA, ALA, or total n-3 FA) exposures in reducing CVD outcomes (incident CVD events including all-cause mortality, CVD mortality, non-fatal CVD events, new diagnosis of CVD, peripheral vascular disease, congestive heart failure, major arrhythmias, and hypertension diagnosis) and specific CVD risk factors (blood pressure, key plasma lipids)?
    - What is the efficacy or association of n-3 FA in preventing CVD outcomes in people
        - Without known CVD (primary prevention)
        - At high risk for CVD (primary prevention), and
        - With known CVD (secondary prevention)?
    - What is the relative efficacy of different n-3 FAs on CVD outcomes and risk factors?
    - Can the CVD outcomes be ordered by strength of intervention effect of n-3 FAs?

2. n-3 FA variables and modifiers:
    - How does the efficacy or association of n-3 FA in preventing CVD outcomes and with CVD risk factors differ in subpopulations, including men, premenopausal women, postmenopausal women, and different age or race/ethnicity groups?
    - What are the effects of potential confounders or interacting factors—such as plasma lipids, body mass index, blood pressure, diabetes, kidney disease, other nutrients or supplements, and drugs (e.g., statins, aspirin, diabetes drugs, hormone replacement therapy)?
    - What is the efficacy or association of different ratios of n-3 FA components in dietary supplements or biomarkers, on CVD outcomes and risk factors?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by ratios of different n-3 FAs—DHA, EPA, and ALA, or other n-3 FAs?
    - How does the efficacy or association of n-3 FA on CVD outcomes and risk factors differ by source (e.g., fish and seafood, common plant oils (e.g., soybean, canola), fish oil supplements, fungal-algal supplements, flaxseed oil supplements)?

- How does the ratio of n-6 FA to n-3 FA intakes or biomarker concentrations affect the efficacy or association of n-3 FA on CVD outcomes and risk factors?
- Is there a threshold or dose-response relationship between n-3 FA exposures and CVD outcomes and risk factors? Does the study type affect these relationships?
- How does the duration of intervention or exposure influence the effect of n-3 FA on CVD outcomes and risk factors?
- What is the effect of baseline n-3 FA status (intake or biomarkers) on the efficacy of n-3 FA intake or supplementation on CVD outcomes and risk factors?

3. Adverse events:
   - What adverse effects are related to n-3 FA intake or biomarker concentrations (in studies of CVD outcomes and risk factors)?
   - What adverse events are reported specifically among people with CVD or diabetes (in studies of CVD outcomes and risk factors)?

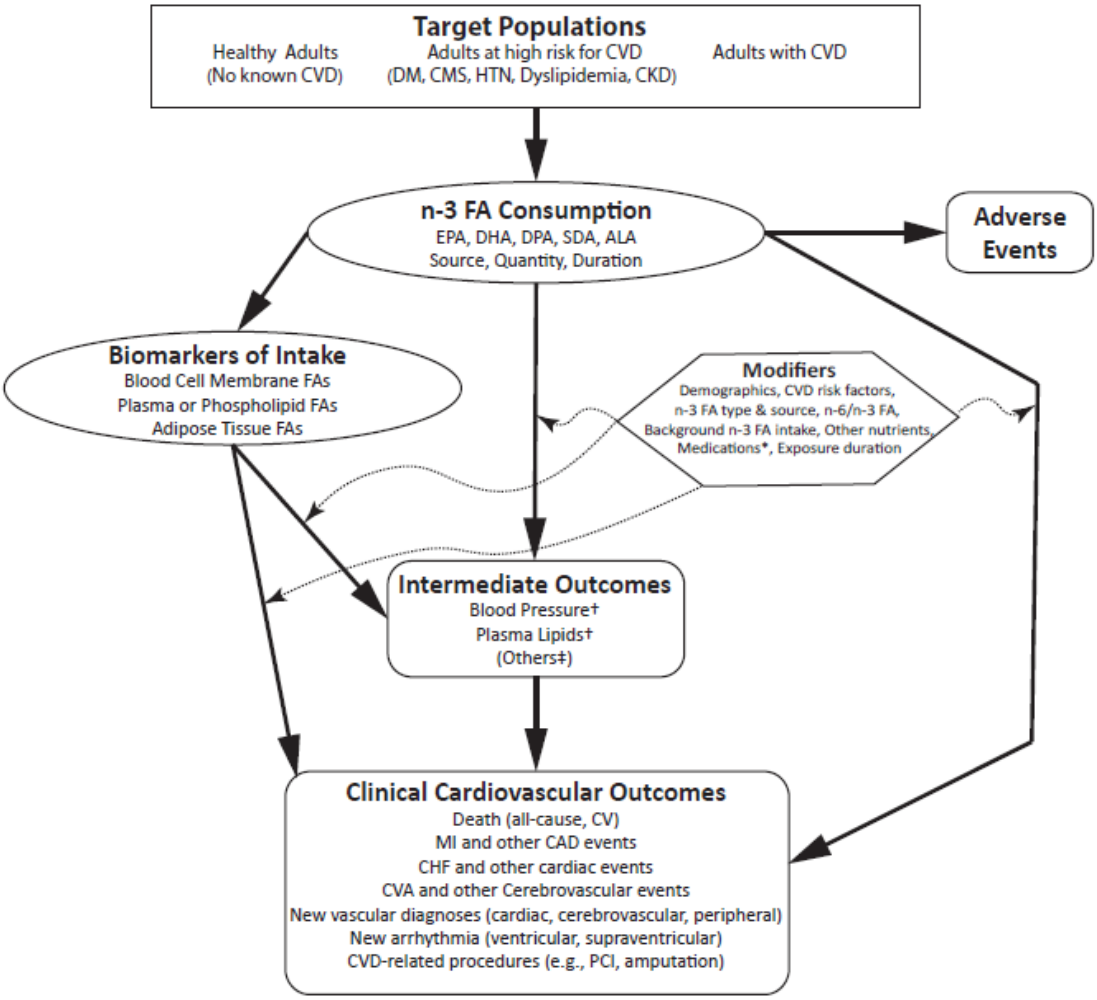
III. Analytic Framework

To guide the assessment of studies that examine the association between n-3 FA intake and cardiovascular outcomes, the analytic framework maps the specific linkages associating the populations of interest, the exposures, modifying factors, and outcomes of interest (**Figure**). The framework graphically presents the key components of well-formulated study questions:

1) Who are the participants (i.e., what is the population and setting of interest, including the diseases or conditions of interest)?
2) What are the interventions?
3) What are the outcomes of interest (intermediate and health outcomes)?
4) What study designs are of value?

Specifically, this analytic framework depicts the chain of logic that evidence must support to link the intervention (exposure to n-3 FA) to improved health outcomes.

Figure. Analytic framework for omega-3 fatty acid exposure and cardiovascular disease.



This framework concerns the effect of n-3 FA exposure (as a supplement or from food sources) on CVD and cardiovascular risk factors. Populations of interest are noted in the top rectangle, exposure in the oval, outcomes in the rounded rectangles, and effect modifiers in the hexagon.

* Specifically, cardiovascular medications, statins, antihypertensives, diabetes medications, hormone replacement regimens.
† Systolic blood pressure, diastolic blood pressure, mean arterial pressure, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides.
‡ Many other intermediate outcomes are likely in the causal pathway between n-3 FA intake and cardiovascular outcome, but only blood pressure and plasma lipids are included in the review.

ALA = alpha-linolenic acid, CAD = coronary artery disease, CHF = congestive heart failure, CKD = nondialysis-dependent chronic kidney disease, CMS = cardiometabolic syndrome, CVA = cerebrovascular accident (stroke), CVD = cardiovascular disease, DHA = docosahexaenoic acid, DM = diabetes mellitus, DPA = docosapentaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, HTN = hypertension, MI = myocardial infarction, n-3 = omega-3, n-6 = omega-6, PCI = percutaneous coronary intervention, SDA = stearidonic acid.

IV. Methods

The present review evaluates the effects of and the associations between n-3 FA (EPA, DPA, ALA and n-3 biomarkers) and CVD outcomes. The Evidence-based Practice Center (EPC) will conduct the review based on a systematic review of the published scientific literature using established methodologies as outlined in the Agency for Healthcare Research and Quality's (AHRQ) Methods Guide for Comparative Effectiveness Reviews (35).

The review is conducted in parallel with a systematic review of n-3 FA and child and maternal health, conducted by another EPC. Several aspects of the review are being coordinated, including eligibility criteria regarding interventions and exposures, search strategies, structure of the reviews, and assessments of the studies' risk of bias, strength of the bodies of evidence, and extraction of study characteristics needed to assess causality.

A. Eligibility Criteria

The currently proposed eligibility criteria are mostly similar to the criteria used in the original 2004 review. The populations remain the same. The interventions and exposures have been expanded to include n-3 FA biomarkers. The list of CVD outcomes of interest has been expanded. Similar study designs will be included. Because some researchers who published studies that were included in the 2004 reviews have been called into question for possible misconduct but have not formally withdrawn their publications (36,37), we will exclude these studies and newly identified related studies (including those written by the same lead authors).

For all Key Questions, the eligibility criteria used will be:

Populations

- Healthy adults (≥18 yr) without CVD or with low to intermediate risk for CVD
- Adults at high risk for CVD (e.g., with diabetes, cardiometabolic syndrome, hypertension, dyslipidemia, non-dialysis chronic kidney disease)
- Adults with clinical CVD (e.g., history of myocardial infarction, angina, transient ischemic attacks)
- Exclude populations chosen for having a non-CVD or non-diabetes-related disease (e.g., cancer, gastrointestinal disease, rheumatic disease, dialysis)

Interventions/Exposures

- n-3 FA supplements
- n-3 FA supplemented foods (e.g., eggs)
- n-3 FA content in diet (e.g., from food frequency questionnaires)
- Biomarkers of n-3 FA intake

- n-3 content of food or supplements must be quantified (e.g., exclude fish diet studies where only servings/week defined, Mediterranean diet studies without n-3 quantified). n-3 quantification can be of total n-3 FA, of a specific n-3 FA (e.g., ALA) or of combined EPA+DHA ("marine oil").
- Exclude n-3 FA dose ≥6 g/day (except for adverse events)

- Exclude weight loss interventions

Comparators
- Placebo or no n-3 FA intervention
- Different n-3 FA source intervention
- Different n-3 FA concentration intervention
- Different n-3 FA dietary exposure (e.g., comparison of quantiles)
- Different n-3 FA biomarker levels (e.g., comparison of quantiles)

Outcomes
- All-cause mortality
- Cardiovascular, cerebrovascular, and peripheral vascular events:
    - Fatal vascular events (e.g., due to myocardial infarction, stroke)
    - Non-fatal vascular events (e.g., myocardial infarction, stroke/CVA, TIA, unstable angina)
    - Coronary heart disease, new diagnosis
    - Congestive heart failure, new diagnosis
    - Cerebrovascular disease, new diagnosis
    - Peripheral vascular disease, new diagnosis
    - Ventricular arrhythmia, new diagnosis
    - Supraventricular arrhythmia, new diagnosis
    - Major vascular interventions/procedures (e.g, revascularization, thrombolysis, lower extremity amputation, defibrillator placement)
- Major CVD risk factors (intermediate outcomes):
    - Blood pressure (new-onset hypertension, systolic, diastolic, and mean arterial pressure)
    - Key plasma lipids (i.e., high density lipoprotein cholesterol [HDL-c], low density lipoprotein cholesterol [LDL-c], total/HDL-c ratio, LDL-c/HDL-c ratio, triglycerides)
- Adverse events (eg, bleeding, major gastrointestinal disturbance), only from intervention studies of supplements

Timing
- Clinical outcomes, including new-onset hypertension (all study designs): ≥1 year followup (and intervention duration, as applicable)
- Intermediate outcomes (blood pressure and plasma lipids) (all study designs): ≥1 month followup
- Adverse events (all study designs): no minimum followup

Setting
- Community-dwelling (non-institutionalized) individuals

Study **D**esign
- RCTs (all outcomes)

- Randomized cross-over (XO) studies (blood pressure and plasma lipids, adverse events), minimum washout period to be determined
- Prospective nonrandomized comparative studies (clinical outcomes, adverse events)
- Prospective cohort (single group) studies, where groups are compared based on n-3 FA intake or intake biomarker values (clinical outcomes)
- Exclude: Retrospective or case control studies or cross-sectional studies (but include prospective nested case control studies). Studies must have measure of intake prior to outcome.

- Minimum sample sizes
    - All outcomes: To be determined
        - We will aim for a minimum of about 25 RCTs for each of the blood pressure and plasma lipid outcomes
            - We expect to include at least 50 RCTs
            - We will choose RCTs based on a combination of sample size, whether they report subgroup or interaction analyses, duration of follow-up, and whether marine oils or ALA was investigated
        - We will aim for a minimum of about 10 longitudinal observational studies for each clinical outcome (and hypertension diagnosis) and also for dietary marine oils, dietary ALA, marine oil biomarkers, and ALA biomarkers.
            - We expect to include at least 50 RCTs
            - We will choose RCTs based on a combination of sample size, whether they report subgroup or interaction analyses, duration of follow-up, and whether marine oils or ALA was investigated

- English language publications
- Peer reviewed publications

B. Literature Search

We will conduct literature searches of studies in MEDLINE®, both the Cochrane Central Trials Registry and Cochrane Database of Systematic Reviews, EMBASE, and CAB Abstracts from 2003 onward (to overlap with the last search run for the 2004 reviews). We will search earlier publications back to 2000 for the newly added outcomes and for biomarkers of n-3 FA intake. We will also include all studies from the original reviews that continue to meet eligibility criteria. We will revise the search strategy used in the original reviews to capture new terms for n-3 FA, biomarkers, and additional outcomes. In electronic searches, we will combine terms for n-3 FA (and biomarkers), CVD and risk factors (blood pressure and plasma lipids), limited to humans, English language, and relevant research designs. Titles and abstracts will be screened to identify articles relevant to each Key Questions. We will also review reference lists of related systematic reviews and selected narrative reviews and primary articles. We will invite

TEP members to provide additional citations. In addition, a call for potentially relevant articles will be posted on the Federal Register (in lieu of Scientific Information Packets). The search will be updated upon submission of the draft report for peer and public review. The **Appendix** displays the current complete search strategy.

All citations found by literature searches will be independently screened by two researchers. Upon the start of citation screening, we will implement a training session where all researchers screen the same articles and conflicts will be discussed. We will iteratively continue training until we have reached agreement regarding the nuances of the eligibility criteria for screening. During double-screening, we will resolve conflicts as a group. All screening will be done in the open-source, online software Abstrackr (http://abstrackr.cebm.brown.edu/).

C. Data Extraction and Management

Each study will be extracted by one methodologist. The extraction will be reviewed and confirmed by at least one other experienced methodologist. Any disagreements will be resolved by discussion among the team. Data will be extracted into customized forms in Systematic Review Data Repository (SRDR) online system (http://srdr.ahrq.gov) and Excel spreadsheets, each designed to capture all elements relevant to the Key Questions. Upon completion of the review, the Excel spreadsheets (of observational study results data) will be uploaded into SRDR and the database will be made accessible to the general public (with capacity to read, download, and comment on data). The basic elements and design of these forms will be the similar to those we have used for other comparative effectiveness reviews, and will include elements that address population characteristics; descriptions of the interventions, exposures, or biomarker status (and comparators) analyzed; outcome definitions; enrolled and analyzed sample sizes; study design features; results; and risk of bias assessment. The form will be developed off the forms used for the original review. We will also include questions pertinent to issues related to causality. We will test the forms on several studies and revise as necessary before full data extraction. All eligible studies from all sources (the new literature search, existing systematic reviews and the original CER) will be fully extracted and entered into SRDR.

D. Assessment of Methodological Risk of Bias of Individual Studies

We will assess the methodological quality of each study based on predefined criteria. For RCTs, we will use the Cochrane risk of bias tool (38), which asks about risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential biases. For observational studies, we will use relevant questions from the Newcastle Ottawa Scale (39). We will also include nutrition study specific risk of bias questions (e.g., related to uncertainty of dietary assessment measurements (40-42). Any quality issues pertinent to specific outcomes within a study will be noted and applied to those outcomes. Any quality issues pertinent to specific outcomes within a study will be noted and considered when determining the overall strength of evidence for conclusions related to those outcomes .

E. Data Synthesis

All included studies will be summarized in narrative form and in summary tables that tabulate the important features of the study populations, design, intervention, outcomes, and results. We plan to build off of and improve on the tables used in the

original review. These included descriptions of the study design, sample size intervention(s), follow-up duration, outcomes, and study quality.

We will analyze different study designs separately, and if appropriate, together, and will compare and contrast populations, exposures, and results across study designs. We will examine any differences in findings between observational and intervention studies. We will evaluate the risk of bias factors as possible explanations for any heterogeneity.

We expect to conduct random effects model meta-analyses of comparative studies, if they are sufficiently similar in population, interventions, and outcomes. If appropriate data are available, we may also conduct meta-regression analyses to evaluate study features, in particular to evaluate dose-response. We will explore subgroup differences within (and possibly across) studies based on the list of comparisons described in the Key Questions.

Dose-response meta-analyses that also allow for threshold effects require specialized approaches because one has to be able to: a) allow the slope of the relationship to change above a threshold which is not necessarily examined in the studies, b) utilize information from adjusted analyses (not simply the unadjusted counts), c) use all levels of exposure in each study (not only extreme levels of intake), d) account for the grouping of data by study, and e) allow for different baseline risks in each study, and for between-study heterogeneity. We plan to analyze separate dose-relationships for each specific n-3 FA (or combination of n-3 FAs, depending on what is described in studies), all n-3 FA (ALA+ EPA+DHA±DPA±SDA), and intake biomarkers with clinical outcomes.

We will compile an appendix table with data related to possible causality criteria. The list of items in this table was compiled based on discussions between the EPCs and ODS after discussion the Bradford Hill criteria (33) and other issues related to determining causality. The table will include a listing of included studies with their population category (healthy, at high CVD risk, with CVD), CVD risk type (e.g., diabetes, hypertension, chronic kidney disease, dyslipidemia), demographics (age, sex, race), cardiovascular history, cardiovascular risk factors (blood pressure, plasma lipids, weight), baseline n-3 intake, n-3 source, n-3 type, how n-3 intake measured, study design (e.g., RCT, prospective or retrospective longitudinal cohort, or other design), exposure duration, followup duration, outcomes reported, effect sizes, difference in n-3 intake (between low and high intake groups), and a dose-corrected effect size.

F. Grading the Strength of Evidence

We will grade the strength of the body of evidence as per the AHRQ methods guide on assessing the strength of evidence (43). We plan to assess the strength of evidence for each outcome. Following the standard AHRQ approach, for each intervention and comparison of intervention, and for each outcome, we will assess the number of studies, their study designs, the study limitations (i.e., risk of bias and overall methodological quality), the directness of the evidence to the Key Questions, the consistency of study results, the precision of any estimates of effect, the likelihood of reporting bias, and the overall findings across studies. Based on these assessments, we will assign a strength of evidence rating as being either high, moderate, or low, or there being insufficient evidence to estimate an effect. The data sources, basic study characteristics, and each strength-of-evidence dimensional rating will be summarized in a

"Summary of Evidence Reviewed" table detailing our reasoning for arriving at the overall strength of evidence rating (see **Table 1** as an example).

Characteristics of observational studies will be abstracted to enable assessment of causality. The relevant characteristics are listed in **Table 2**.

G. Assessing Applicability

We will assess the applicability within and across studies with reference to whether people in the studies are in the three populations of interest (healthy, at risk, and with CVD), and as pertains to n-3 FA source, type, and dose/exposure.

Table 1. Strength of evidence Domains.
(Separate tables for evidence on different n-3 FAs and different populations [healthy, at risk, and with CVD])

Outcome **Strength of Evidence Grade**	**Study Design: No. Studies (N)**	**Study Limitations**	**Directness**	**Consistency**	**Precision**	**Reporting Bias**	**Other Issues**	**Findings**
Outcome 1 High, Moderate, Low, or Insufficient	RCTs: No. (N) Observational: No. (N)	Low, Medium, or High	DIrect or Indirect	Consistent or Inconsistent	Precise or Imprecise	Undetected or Suspect	None or Other Issues	Qualitative (and quantitative) summary of findings

Table 2. Study Level Details Related to Causality

Study	Study years	Country	Population	Sample size (total)	Age	Sex	Race	Medical History	Risk type	Blood pressure	Lipids	Weight	Baseline n-3 intake	n-3 source	n-3 type(s)	n-3 measure	Study design	Outcome	Reported effect Size	Dose/intake difference	Dose-corrected effect size

Options for Population: Healthy, At risk (for CVD), CVD (cardiovascular disease).
Options for Risk type: DM (diabetes), HTN (hypertension), CKD (chronic kidney disease), Dyslipidemia.
Options for n-3 (omega-3 fatty acid) measure: defined (supplement), defined (biomarker), estimated (food item), estimated (food frequency questionnaire).

V. References

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VI. Definition of Terms

Not applicable. All terms are defined above, as needed.

VII. Summary of Protocol Amendments

No protocol amendments to date.

VIII. Review of Key Questions

The key questions will be reviewed and refined as needed by the EPC with input from the Technical Expert Panel (TEP) to assure that the questions are specific and explicit about what information is being reviewed. In addition, the key questions will be posted for public comment and finalized by the EPC after review of the comments.

IX. Key Informants

Key Informants will not be employed for this update to an existing CER.

X. Technical Experts

Technical Experts constitute a multi-disciplinary group of clinical, content, and methodological experts who provide input in defining populations, interventions, comparisons, or outcomes and identify particular studies or databases to search. They are selected to provide broad expertise and perspectives specific to the topic under development. Divergent and conflicting opinions are common and perceived as health scientific discourse that results in a thoughtful, relevant systematic review. Therefore study questions, design, and methodological approaches do not necessarily represent the views of individual technical and content experts. Technical Experts provide information to the EPC to identify literature search strategies and recommend approaches to specific issues as requested by the EPC. Technical Experts do not do analysis of any kind nor do they contribute to the writing of the report. They have not reviewed the report, except as given the opportunity to do so through the peer or public review mechanism.

Technical Experts must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals are invited to serve as Technical Experts and those who present with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

XI. Peer Reviewers

Peer reviewers are invited to provide written comments on the draft report based on their clinical, content, or methodological expertise. The EPC considers all peer review comments on the draft report in preparation of the final report. Peer reviewers do not participate in writing or editing of the final report or other products. The final report does not necessarily represent the views of individual reviewers. The EPC will complete a disposition of all peer review comments. The disposition of comments for systematic reviews and technical briefs will be published three months after the publication of the evidence report.

Potential Peer Reviewers must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Invited Peer Reviewers may not have any financial conflict of interest greater than $10,000. Peer reviewers who disclose potential business or professional conflicts of interest may submit comments on draft reports through the public comment mechanism.

XII. EPC Team Disclosures

EPC core team members must disclose any financial conflicts of interest greater than $1,000 and any other relevant business or professional conflicts of interest. Related financial conflicts of interest that cumulatively total greater than $1,000 will usually disqualify EPC core team investigators. The EPC core team members have not financial or other conflicts to report.

XIII. Role of the Funder

This project was funded under Contract No. HHSA 290 2012 00012 I from the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services. The Task Order Officer reviewed contract deliverables for adherence to contract requirements and quality. The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

Appendix

Omega 3 CVD update 2015-update search
Databases: Medline, Cochrane databases, CAB abstracts; equivalent searches done in EMBASE 10/9/2014

Search 1 (updated outcomes, limited to 2002-2015)

#	Search	
1.	exp fatty acids, omega-3/	Omega 3 terms
2.	((omega-3 or omega 3 or omega3) and fatty acid$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
3.	fatty acids, essential/	
4.	linolenic acids/	
5.	exp fish oils/	
6.	((n 3 or n3 or n-3) and (oil$ or pufa or fatty acid$ or omega 3)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
7.	Docosahexaenoic Acids/	
8.	docosahexa?noic.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
9.	Eicosapentaenoic Acid/	
10.	eicosapenta?noic.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
11.	icosapent?enoic.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
12.	(alpha linolenic or alphalinolenic or alpha-linolenic).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
13.	(linolenate or cervonic or timnodonic or stearidonic).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
14.	menhaden oil$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
15.	((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed or perilla or shiso) adj2 oil$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
16.	(walnut$ or butternut$ or soybean$ or pumpkin seed$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
17.	(fish adj2 oil$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
18.	(cod liver oil$ or codliver oil$ or marine oil$ or marine fat$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
19.	(salmon or mackerel or herring or tuna or halibut or seaweed or anchov$ or sardine$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
20.	(Ropufa or MaxEPA or Omacor or Efamed or ResQ or Epagis or Almarin or Coromega or Lovaza or Vascepa or icosapent ethyl).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
21.	(fish consumption or fish intake or (fish adj2 diet$)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
22.	(mediterranean adj diet$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
23.	((red blood cell or phospholipid or plasma fatty acid or plasma or phospholipid or triacylglycerol or cholesteryl or ester or adipos$ or fatty acid or erythrocyte or ghost or platelet or granulocyte or neutrophil or mononuclear or LDL or HDL) and (DHA or docosahexa?noic or EPA or eicosapenta?noic or SDA or linolenic or stearidonic or omega)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	n-3 Biomarkers
24.	or/1-23	n-3
25.	exp cardiovascular diseases/	Cardiovascular diseases, risk factors, adverse events
26.	atherosclero$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
27.	Arteriosclero$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
28.	cardioprotect$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
29.	Coronary.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
30.	heart disease$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
31.	Myocardial infarct$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
32.	exp Cerebrovascular Accident/	
33.	stroke.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
34.	(Transient Ischemic Attack or TIA).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
35.	exp lipids/	
36.	lipid$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
37.	exp cholesterol/	
38.	cholesterol.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
39.	exp Lipoproteins, LDL/	
40.	exp Lipoproteins, HDL/	
41.	exp triglycerides/	
42.	triglycerides.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
43.	exp Hyperlipidemias/	

#	Search	
44.	hypertriglyceridem$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
45.	hyperlipidemia$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
46.	exp dyslipidemias/	
47.	dyslipidemia$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
48.	exp blood pressure/	
49.	blood pressure.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
50.	(diastol$ or systol$ or mean arterial).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
51.	exp hypertension/	
52.	hypertension.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
53.	exp Hemorrhage/	
54.	hemorrhag$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
55.	bleeding.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
56.	or/25-55	
57.	24 and 56	**n-3 & CVD**
58.	(random$ or rct$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	**Study designs**
59.	exp randomized controlled trials/	
60.	exp Randomized Controlled Trials as Topic/	
61.	exp random allocation/	
62.	exp double-blind method/	
63.	exp single-blind method/	
64.	randomized controlled trial.pt.	
65.	clinical trial.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
66.	(clin$ adj trial$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
67.	((singl$ or doubl$ or trebl$ or tripl$) adj (blind$ or mask$)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
68.	exp placebos/	
69.	placebo$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
70.	randomly allocated.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
71.	(allocated adj2 random$).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
72.	comparative study.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
73.	follow-up studies/	
74.	(follow up or followup).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
75.	exp case-control studies/	
76.	(case adj20 control).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
77.	exp longitudinal studies/	
78.	longitudinal.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
79.	exp cohort studies/	
80.	cohort.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
81.	exp prospective studies/	
82.	exp evaluation studies/	
83.	(observational adj (study or studies)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
84.	food frequency questionnaire$.mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
85.	or/58-84	
86.	57 and 85	**n-3, CVD, Designs**
87.	limit 86 to (addresses or autobiography or bibliography or biography or case reports or comment or congresses or dictionary or directory or editorial or festschrift or government publications or historical article or interview or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or periodical index)	**Not non-studies**
88.	86 not 87	
89.	limit 88 to english language	**Limits**
90.	limit 89 to humans	
91.	(guidelines or practice guideline or meta analysis or systematic review).pt.	**SRs, GLs**
92.	(systematic$ adj3 review$).tw.	
93.	91 or 92	
94.	57 and 93	
95.	limit 94 to yr="2002 - 2015"	**Non-SRs**
96.	90 not 94	**SRs**
97.	limit 96 to yr="2002 - 2015"	

Search 2 (new outcomes and biomarkers, limited to 2000-2015)
[Only difference is new outcomes and publication dates]

#	Search	
1.	exp fatty acids, omega-3/	
2.	((omega-3 or omega 3 or omega3) and fatty acid$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
3.	fatty acids, essential/	
4.	linolenic acids/	
5.	exp fish oils/	
6.	((n 3 or n3 or n-3) and (oil$ or pufa or fatty acid$ or omega 3)).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
7.	Docosahexaenoic Acids/	
8.	docosahexa?noic.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
9.	Eicosapentaenoic Acid/	
10.	eicosapenta?noic.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
11.	icosapent?enoic.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
12.	(alpha linolenic or alphalinolenic or alpha-linolenic).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
13.	(linolenate or cervonic or timnodonic or stearidonic).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
14.	menhaden oil$.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
15.	((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed or perilla or shiso) adj2 oil$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
16.	(walnut$ or butternut$ or soybean$ or pumpkin seed$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
17.	(fish adj2 oil$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
18.	(cod liver oil$ or codliver oil$ or marine oil$ or marine fat$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
19.	(salmon or mackerel or herring or tuna or halibut or seaweed or anchov$ or sardine$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
20.	(Ropufa or MaxEPA or Omacor or Efamed or ResQ or Epagis or Almarin or Coromega or Lovaza or Vascepa or icosapent ethyl).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
21.	(fish consumption or fish intake or (fish adj2 diet$)).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
22.	(mediterranean adj diet$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
23.	((red blood cell or phospholipid or plasma fatty acid or plasma or phospholipid or triacylglycerol or cholesteryl or ester or adipos$ or fatty acid or erythrocyte or ghost or platelet or granulocyte or neutrophil or mononuclear or LDL or HDL) and (DHA or docosahexa?noic or EPA or eicosapenta?noic or SDA or linolenic or stearidonic or omega)).mp. [mp=ti, ab, ot, nm, hw, kf, px, rx, ui, tx, kw, ct, sh, bt, id, cc]	
24.	or/1-23	
25.	(random$ or rct$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
26.	exp randomized controlled trials/	
27.	exp Randomized Controlled Trials as Topic/	
28.	exp random allocation/	
29.	exp double-blind method/	
30.	exp single-blind method/	
31.	randomized controlled trial.pt.	
32.	clinical trial.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
33.	(clin$ adj trial$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
34.	((singl$ or doubl$ or trebl$ or tripl$) adj (blind$ or mask$)).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
35.	exp placebos/	
36.	placebo$.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
37.	randomly allocated.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
38.	(allocated adj2 random$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
39.	comparative study.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
40.	follow-up studies/	
41.	(follow up or followup).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
42.	exp case-control studies/	
43.	(case adj20 control).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
44.	exp longitudinal studies/	
45.	longitudinal.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
46.	exp cohort studies/	
47.	cohort.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
48.	exp prospective studies/	
49.	exp evaluation studies/	
50.	(observational adj (study or studies)).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
51.	Cross-Sectional Studies/	
52.	(cross section$ or cross-section$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	

53.	food frequency questionnaire$.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
54.	or/25-53	
55.	24 and 54	
56.	exp heart failure/	
57.	Heart failure$.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
58.	exp pulmonary edema/	
59.	pulmonary edema.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
60.	pulmonary oedema.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
61.	(ejection adj2 fraction).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
62.	exp peripheral vascular diseases/	
63.	(peripheral and vascular and disease$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
64.	claudication.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
65.	exp arrhythmias, cardiac/	
66.	(arrhythmi$ or Antiarrhythmi$).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
67.	Fibrillation.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
68.	Flutter.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
69.	exp tachycardia/	
70.	tachycardia.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
71.	tachyarrhythmia.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
72.	exp bradycardia/	
73.	bradycardia.mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
74.	exp death, sudden/	
75.	(sudden adj death).mp. [mp=ti, ot, ab, nm, hw, kw, kf, px, rx, ui, an, tx, sh, ct, bt, id, cc]	
76.	or/56-75	
77.	24 and 54 and 76	
78.	limit 77 to (addresses or autobiography or bibliography or biography or case reports or comment or congresses or dictionary or directory or editorial or festschrift or government publications or historical article or interview or lectures or legal cases or legislation or letter or news or newspaper article or patient education handout or periodical index)	
79.	77 not 78	
80.	limit 79 to english language	
81.	limit 80 to humans	
82.	(guidelines or practice guideline or meta analysis or systematic review).pt.	
83.	(systematic$ adj3 review$).tw.	
84.	82 or 83	
85.	24 and 76 and 84	
86.	81 not 85	